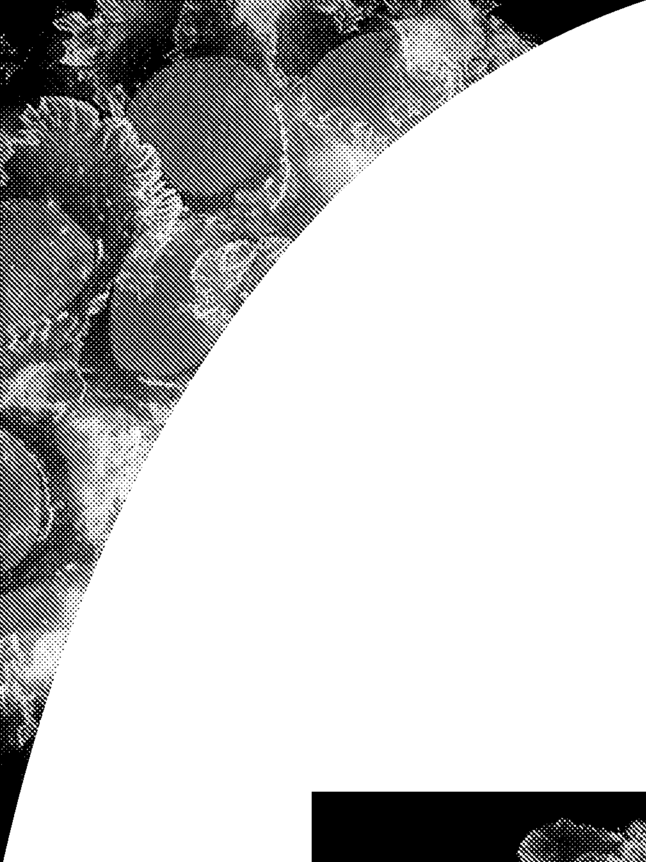
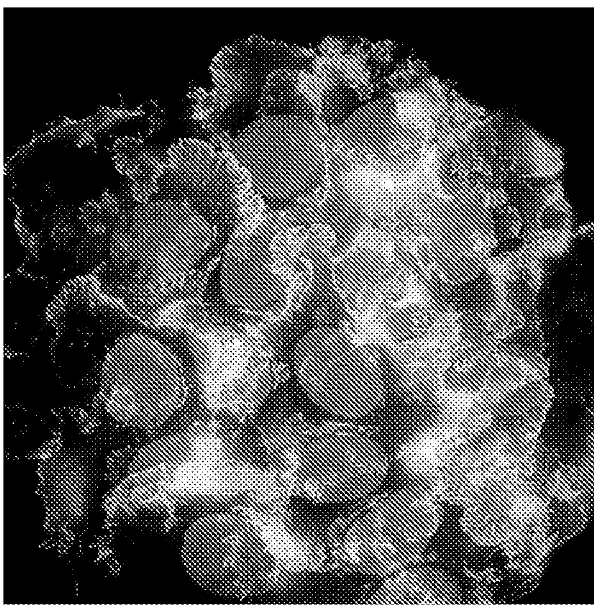


ATCC® cultures™

Maintaining High Standards in Cell Culture



ATCC produces and distributes over 3,600 cell lines to more than 80 countries.

Learn more about our work and why it's essential to yours.

Your **Discoveries** Begin With **Us.**®

EXHIBIT E

ATCC is a unique, nonprofit life science company committed to the authentication, collection, preservation and distribution of living cultures of microorganisms, viruses and cell lines.

Founded in 1925, ATCC was entrusted with its first cell line in 1962 (ATCC® CCL-1™) and has consistently attained the highest standards and used the most reliable procedures to provide verification of every cell line since.

As the use of cell cultures has expanded, the number of reported cases of problems associated with poor cell-culture practices has also increased.[2,4,9,10,19,20,21,22,25] In numerous cases, aberrations and contamination in commonly used laboratory stocks have led to spurious results.[1,3,5,6,8,9,18,28,29]

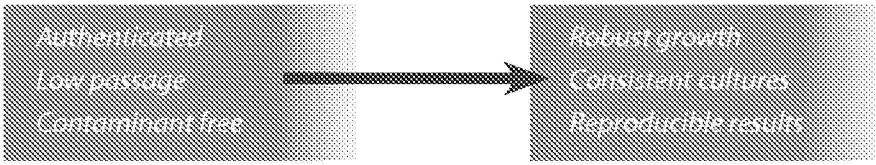
The scientific community is increasingly recognizing that cell line integrity is critical for maintaining high standards in research. Initiatives have called for standardized cell culture quality, including confirmation of cell line identity (through authentication), as a condition for receipt of grant funds from major agencies (NIH, NSF, HHMI, ACS, etc.) as well as for publication of research using cultured cells.[31,32,33]

The comprehensive tests required to ensure these standards are costly, time-consuming and require a high level of expertise. For years, scientists worldwide have relied on ATCC to provide fully authenticated and contamination-free biological reagents as a cost-effective and reliable option.

This document reviews the systematic processes and comprehensive testing used at ATCC to maintain high standards for cell line identity and integrity.

ATCC accessioning — acquiring new cell lines

ATCC cell lines are provided with comprehensive and repeated authentication and contamination checking — starting with the depositor's original material and continuing through the production of vials for distribution — ensuring that delivered materials meet the highest standards and expectations.



The general ATCC cell line accessioning scheme encompasses a series of tests which confirm the identity of a cell line and ensure that it is free of contamination.

A systematic seed-stock cell-banking method is used to produce virtually identical distribution lots, ensuring consistent materials for every order.

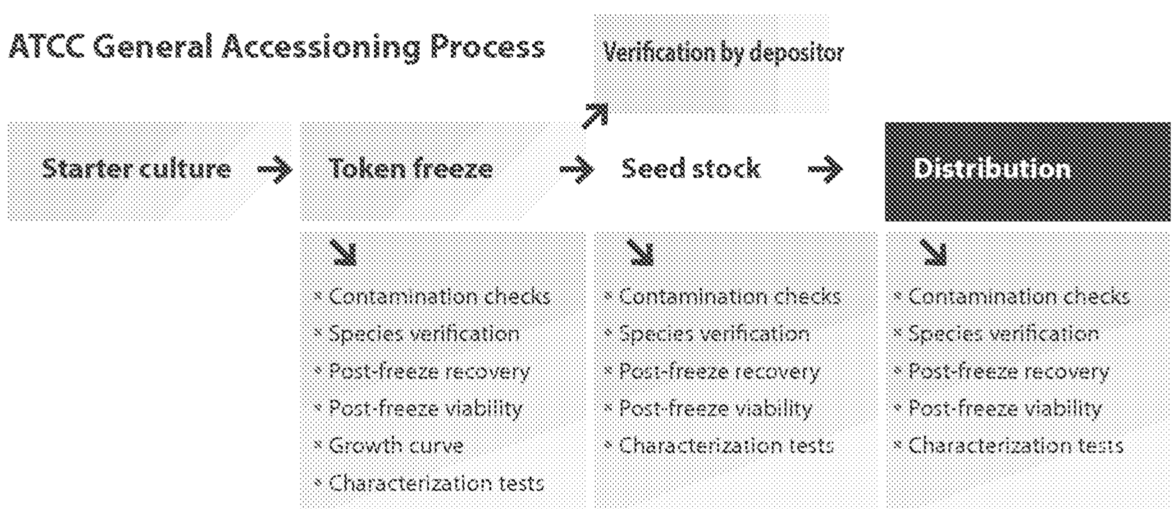


Figure 1 • The general ATCC Accessioning Process includes many tests that are repeated at every stage to provide cell line identity verification and unsurpassed quality-testing for all bioproduction runs.

ATCC authentication — verifying cell lines

Experimental success corresponds directly to the quality and conditions of cell lines used. Cells that are kept too long in culture and are not periodically tested for genotypic or phenotypic stability may no longer be reliable models of the original source material.

To maintain high cell culture standards and ensure reliable, reproducible results, the use of authenticated and quality-tested cell lines from a recognized cell bank is highly recommended.

ATCC authenticates cell lines routinely with the following tests:

■ Short tandem repeat (STR) profiling establishes a DNA fingerprint for human cell lines. ATCC STR profiling uses multiplex PCR to simultaneously amplify the amelogenin gene and eight of the most informative polymorphic markers in the human genome. The pattern of repeats results in a unique STR identity profile for each cell line analyzed. STR analysis is critical for verifying the identity of human cell lines and is performed for each distribution lot. The results are compared to the baseline profile of the token stock derived from the depositor.

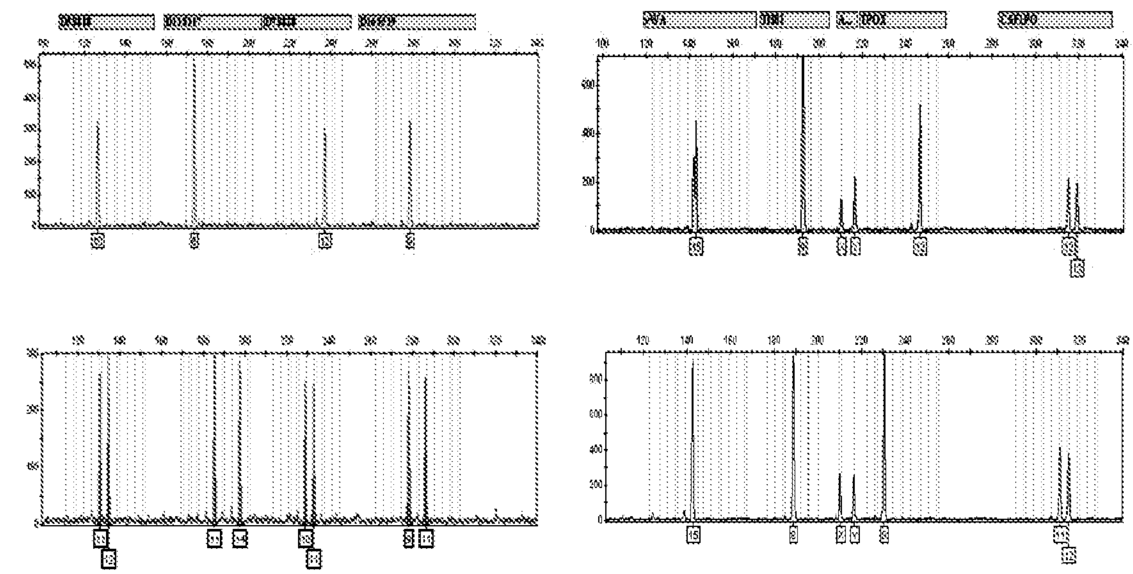


Figure 2 • STR profile of two unrelated cell lines. Top: KU812E (ATCC® CRL-2100™). Bottom: MRC-5 (ATCC® CCL-171™). Amplicons are generated using Promega PowerPlex® 1.2 system, separated by electrophoresis and analyzed using Genotyper® 2.0 software from Applied Biosystems.

"Evidence suggests that up to one-third of tumor cell lines being used in scientific research are affected by inter- or intraspecies cross-contamination or have been wrongly identified, thereby rendering many of the conclusions doubtful if not completely invalid."

Lancet Oncology, vol. 2, July 2001, p. 393

Cell morphology is monitored throughout all ATCC processes. Cellular morphology can vary between lines depending on the health of the cells and, in some cases, the differentiation state — a critical property in certain assays. Morphology can change with plating density as well as with different media and sera combinations. Morphologies of cells grown at low and high densities at ATCC are recorded and used routinely to check cell lines during accessioning and bioproduction.

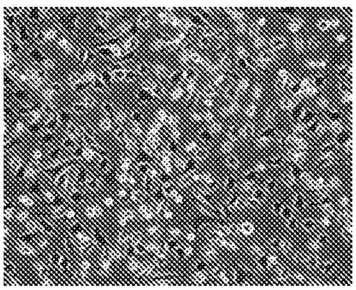


Figure 3 • ATCC® CCL-1™ at high cell density

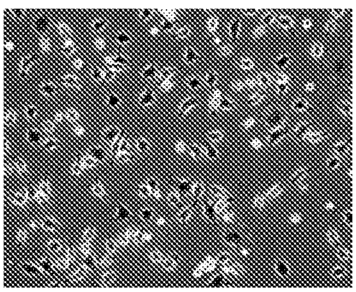


Figure 4 • ATCC® CCL-1™ at low cell density

Karyotyping is performed to identify the species as well as variation within the cell line. Karyotyping is a basic and indispensable test performed routinely to determine if the line has maintained a stable genotype. Karyotyping is performed on many ATCC classic cell lines and all embryonic stem cell lines.

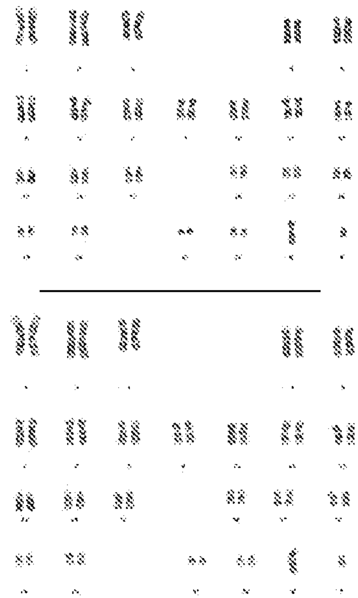


Figure 5 • ATCC® CRL-4001™ Giemsa-banding on distribution (top) and seed (bottom) stocks.

Isoenzyme analysis is used to verify the species of origin. Isoenzyme specimens are differentiated based on electrophoretic properties. Using the results, ATCC can verify information from the depositor regarding the source species of a cell line and check for species cross-contamination. Isoenzyme analysis is part of ATCC accessioning and each distribution lot is assayed to verify the species.

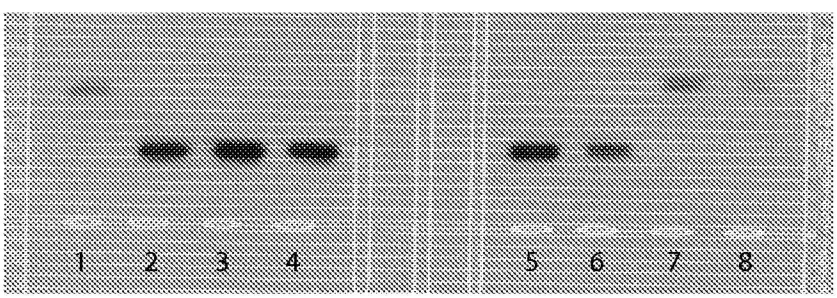


Figure 6 • Isoenzymology analysis of nucleoside phosphorylase on cell line ATCC® CRL-4000™. Lanes: 1 mouse control; 2 human control; 3 token freeze; 4 seed stock; 5 distribution stock; 6 distribution stock after 15 doublings; 7 and 8 mouse controls.

ATCC contamination tests

ATCC performs rigorous and repeated testing to ensure that cell cultures are free of mycoplasma or other bacterial or fungal agents. ATCC tests conform to the mycoplasma-testing stipulations recommended by the FDA "Points to Consider" protocol.

Contamination can profoundly affect the following:

- Cell growth and function
- Transfection
- Morphology and differentiation state
- Gene expression

ATCC ensures contamination-free cell lines by testing in duplicate each lot of the following stocks:

- Token
- Seed
- Distribution

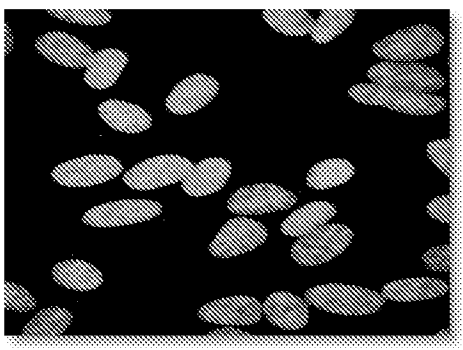


Figure 7 • Hoechst staining of an uncontaminated cell culture. Evenly fluorescent nuclei indicate the absence of mycoplasma.

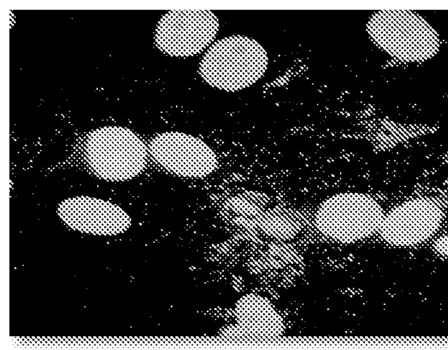


Figure 8 • Hoechst staining of a contaminated cell culture. Contamination is indicated by the extracellular fluorescence.

The damaging effects of mycoplasma contamination on cell lines has been described in detail[6,7,9,11,16,19] and is a major problem in cell culture.[9,15] The problem is exacerbated with the exchange of cell lines between laboratories. Because mycoplasma growth in cell cultures cannot be detected visually or under the microscope, routine testing remains the only assurance against contamination.

ATCC provides consistent, low-passage cultures

ATCC follows a strict seed-stock cell-banking method to ensure distribution of consistent, low passage cell cultures (Figure 1). A large number of frozen vials are prepared from depositor-supplied stock which are then stored as seed stock and used for future production.

Avoiding the use of cell lines that have been in culture too long is a first step to ensuring reliable and reproducible results. Important characteristics can change when cells are cultured for extended periods.[20,22,23,24,26,27,30] Many high-passage laboratory stocks are aberrant to the extent that the cells are no longer reliable models of the original source material.[2,21,25,29]

High-passage cell lines can exhibit alterations in the following properties:

- Morphology
- Growth rates
- Response to stimuli
- Protein expression and signaling

Data shown in figures 9 through 11 describe experimental differences between low- and high-passage cell lines.

The data demonstrate differences in cell differentiation in low-passage and high-passage Caco-2 cells.

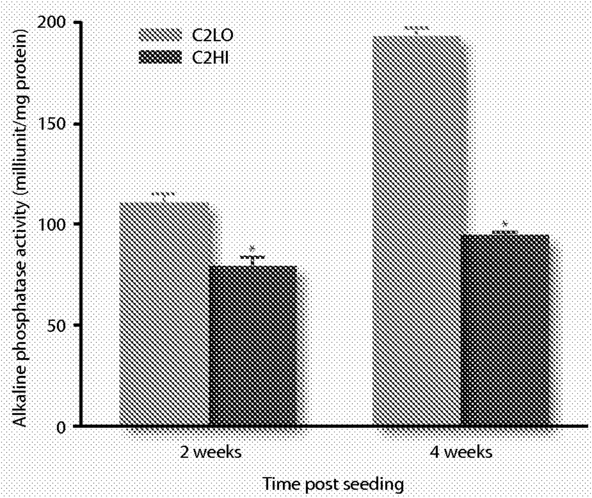


Figure 9 • Alkaline phosphatase activity was reduced to 29% and 67% in high-passage Caco-2 cells (C2HI, passage number 93-108) compared to low-passage cells (C2LO, passage number 28–36) at two and four weeks after seeding, respectively. Alkaline phosphatase activity indicates the lack of cell differentiation. The reduction in activity exhibited by the high-passage cells suggests that the cells are differentiating at a faster rate than the low-passage cells. Reproduced from Yu et al. 1997.

The data demonstrate differences in proliferation and secretion in low- and high-passage LNCaP cells.

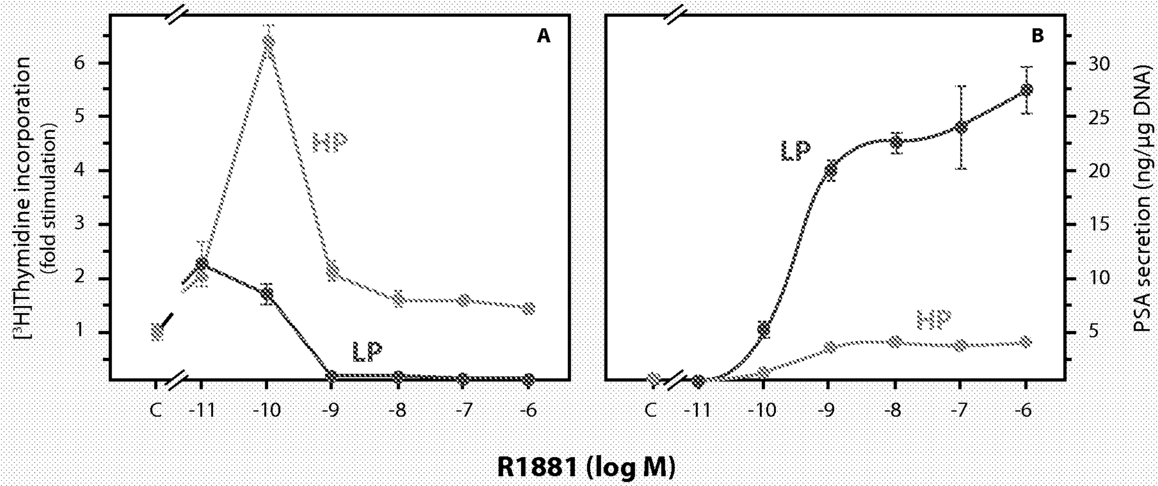
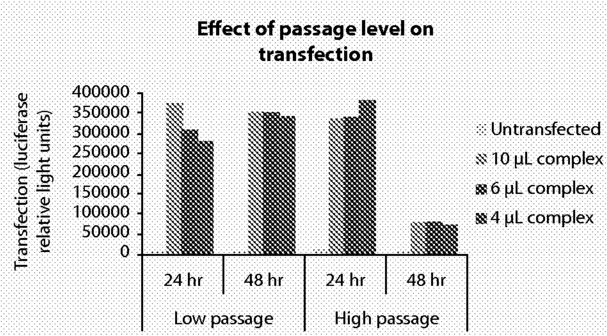


Figure 10 • Two samples of LNCaP prostatic adenocarninoma cells were obtained from ATCC. One sample was passaged 24 times (low passage, LP) and a second sample was passaged approximately 80 times (high passage, HP). [^{3}H]Thymidine incorporation (A) and PSA secretion (B) were measured after three days of incubation with increasing concentrations of the synthetic androgen R1881, as described in Esquenet et al. 1997. With this and other data, the authors concluded: "Low passage and high passage LNCaP cells display markedly divergent responses not only to androgens but also to retinoids."

The data demonstrate low- and high-passage RAW 264.7 (ATCC® TIB-71™) cells transfect equally well, but protein expression is significantly reduced in the high-passage samples.



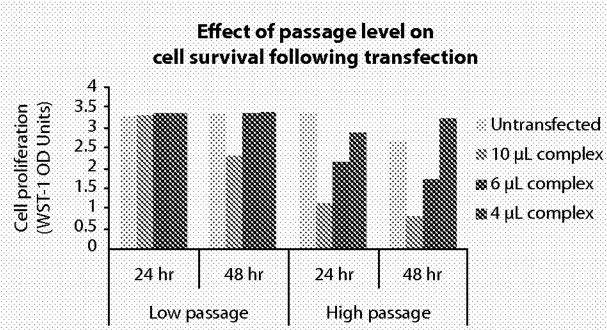


Figure 11 • RAW 264.7 (ATCC® TIB-71™) cells were transfected with a plasmid for luciferase expression at passage number 5 (low passage) and 74 (high passage) using FuGENE® HD Transfection Reagent for comparative studies. Three volumes (4, 6 and 10 µL) of the same complex (5:2 ratio of reagent:DNA) were added to all cells. Similar expression levels (top graph) were observed 24 hours post transfection at either passage number. However, luciferase expression dropped off significantly 48 hours post transfection in the high-passage cells. Minimal inhibition of cell proliferation (bottom graph) was observed in low-passage cells with all three volumes of complex. In contrast, growth inhibition was observed in the high-passage cells when 6 – 10 µL of the complex was added. This effect on proliferation was not observed when less complex was added. (Data supplied by Roche Applied Science.)

Take advantage of the superior quality of ATCC cell lines

ATCC provides two easy ways to find detailed information about the nearly 3,600 cell lines in the ATCC Cell Biology Collection:

- **Use the online search at www.atcc.org**
  - Select "Cell Lines and Hybridomas" from the menu on the left.
  - Enter terms in the provided fields to specify the search or enter a search expression in the full-text search field.
  - Click the name of cell line in the results page to view information.

- **Use the ATCC Cell Biology Catalog**
  - Browse the lists for the cell line and note the ATCC catalog number.
  - Visit the ATCC website at www.atcc.org.
  - Select "Search by ATCC Number" from the drop-down menu at the upper right, and enter the catalog number to view information.

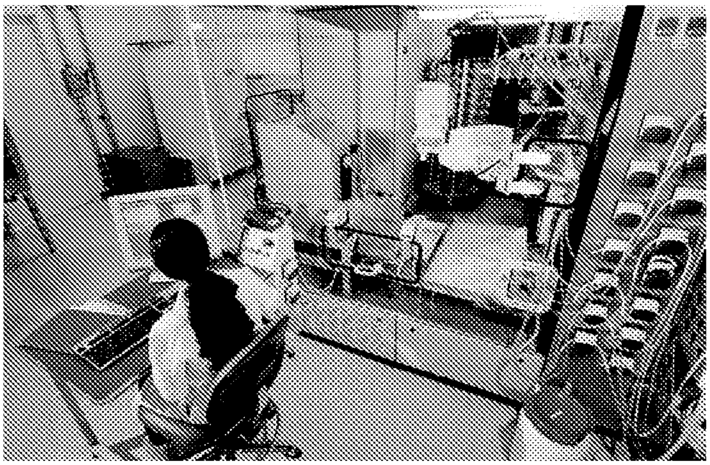


Figure 12 • ATCC routinely uses the SelecT™ system for automated cell culture bioproduction.

Distribute your cell line by depositing with ATCC

Distributing cell lines to colleagues and collaborators can be time-consuming and costly. To ensure that your valuable lines are safely maintained and consistently distributed, consider depositing with ATCC.

When you deposit cultures at ATCC, you are promoting technology transfer by facilitating access to materials for researchers everywhere. ATCC, as the largest and most diverse biological resource center in the world, serves as the custodian of your deposited line; you retain ownership. By including detailed information about the line on the ATCC website, ATCC helps you inform researchers about the availability of the line as well as its special features and characteristics.

Making an ATCC cell line deposit benefits everyone and ensures the integrity of the line for future generations. For information about depositing a cell line, visit the "Make a Deposit" section of the ATCC website or contact ATCC technical services.

If ATCC does not have a cell line you want, send a request to tech@atcc.org.

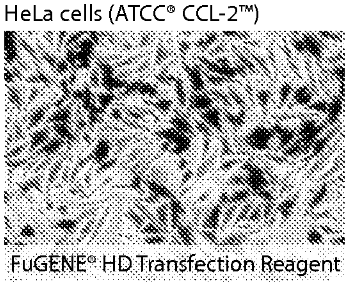
ATCC collaborations — providing consistency to applied science

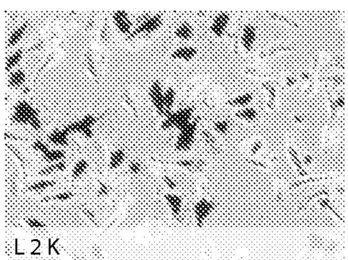
Regarded as standard experimental reagents, cell lines of the highest quality are recommended to ensure reproducible and reliable results from life science products. Manufacturers of quality kits and reagents routinely and exclusively use ATCC cell lines for product development and optimization. Performance of an optimized product can suffer when used with cell lines of inferior quality.

To make it easier to determine the quality of reagents and applications using cell lines, ATCC is working with other life science companies to promote the use of authenticated, quality-tested cell lines by providing access to references, protocols and detailed information about cell cultures and applications.

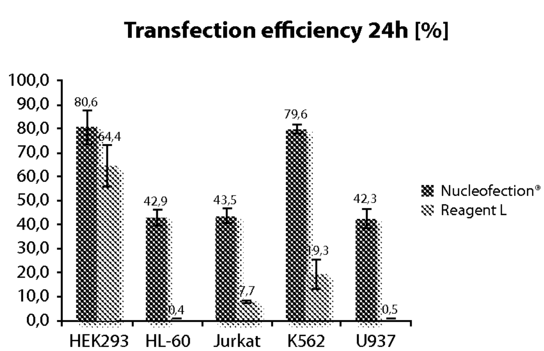
Diagnostics

HeLa cells (ATCC® CCL-2™)





Roche Applied Science set the standard for transfection with FuGENE® 6 Transfection Reagent. With the launch of FuGENE® HD Transfection Reagent, Roche again takes transfection to a higher level, enabling the results needed to advance research. An extensive database of cell lines transfected using these reagents is available at www.roche-applied-science.com/transfection with links to protocols and information regarding the successful transfection of hundreds of ATCC cell lines. Choose transfection reagents from Roche Applied Science combined with fresh, authenticated cell lines from ATCC and move closer to discovery.



amaxa biosystems

amaxa Nucleofector® technology is a well-established method for the transfer into cells of various substrates (e.g., DNA, siRNA, peptides). Novel electrical parameters in combination with cell-type-specific solutions allow the manipulation of cell lines, including primary cells and lines that previously were not amenable to gene transfer. Optimized protocols (e.g., for specific ATCC cell lines) guarantee high transfer efficiencies along with superior cell survival and minimal impact on cell metabolism (www.amaxa.com).

Offering complementary and superior cell transfection solutions, amaxa and Roche Applied Science Web links are found on approved ATCC cell lines.

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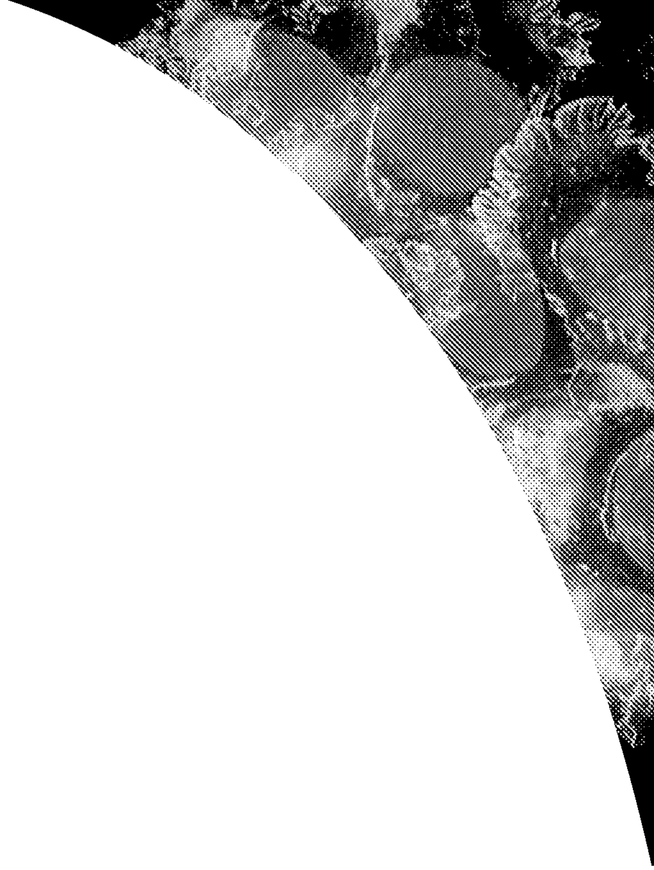
ATCC requests that cell lines acquired from ATCC be referenced in scientific publications with the common name followed by the ATCC catalog number; e.g., NIH/3T3, ATCC® CRL-1658™



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